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SUGAR-AMINO ACID INTERACTION IN THE DIFFUSATE OF WATER EXTRACT OF BEEF AND MODEL SYSTEMS

SUMMARY—Diffusates of water extract of beef and model systems containing amino acids or sugars, or both, in approximately the same concentrations as in beef diffusate were heated at 125°C for up to 60 min. Quantitative analyses of amino acids and sugars were performed. A brothy odor developed on boiling the diffusate (30 min of heating), but there were minor changes in amino acid concentrations except for arginine. After pyrolysis (45 min of heating), roast aroma and brown color developed and there was 40–60% loss of most amino acids. Glucose and fructose concentrations were only slightly affected during boiling, but ribose decreased about 25%. On pyrolysis, sugars completely disappeared. Amino acids heated alone decreased in concentration about 25% and sugars heated alone decreased approximately 20 to 55% on pyrolysis. A sugar-amino acid mixture simulating beef diffusate suffered greater losses in concentrations of amino acids on pyrolysis than did the diffusate. No pattern of changes in amino acid or sugar concentrations was detected to account for the aroma of roast meat on pyrolysis. In beef diffusate, sugars appear to be limiting and a portion of the amino acids remains after pyrolysis. When excess sugar is added, free amino acids disappear completely and the ratio of the amount of glucose disappearing to amino N disappearing levels off at about 4:1.

INTRODUCTION

THE INTERACTION of amino acids and carbohydrates, or α -dicarbonyl compounds, results in the formation of brown pigments. The chemistry of this browning reaction has been covered in a number of reviews (Hodge, 1967; Reynolds, 1963, 1965). Concomitantly with pigments, other products that may be odorous are formed. Since amino acids and requisite reducing compounds are present or can be formed readily in foods, and since browning occurs as a result of heating, the aromas of various food products have been linked to products of the browning reaction (Pinto et al., 1966; Horvat et al.,

1962; Moores et al., 1964; Linko et al., 1963). In meat products, involvement of the Maillard, or browning, reaction with flavor has been mentioned by Hornstein et al. (1960), Wood (1961) and Macy et al. (1964b). However, for the most part this has been based on indirect evidence, as no specific reaction, or reaction product, has been reported to yield a meat aroma. A number of investigators have reported on aromas derived from heating sugars and amino acids under several conditions of temperature and pH. The descriptions of these aromas by Herz et al. (1960), Barnes et al. (1947) Kiely et al. (1960) and Rothe et al. (1963) mention cheese, potato and bakery odors,

but the only reference to a meaty aroma is from the reaction of cysteine and sugar. El'Ode et al. (1966), on the other hand, describe aromas of several amino acids and sugars heated at 180°C in terms of broth or meat. Wood (1961) did obtain a meaty aroma on heating glucose and a mixture of amino acids in the concentrations in which they were present in a meat extract, but quantitative data were not reported. In recent years the only study on the quantitative changes resulting from heating amino acid and sugar constituents in meat extracts has been that of Macy et al. (1964b). These investigators also reported that after heating for 1 hr in a boiling-water bath a typical brothy aroma and a brown color developed in extracts of beef, pork and lamb.

Our studies on meat flavor have been directed toward the characterization of the strong roast beef or broiling steak aroma that develops on the pyrolysis of water extracts of meat (Wasserman et al., 1965). We have reported that these solutions, which give rise to dark-brown pigments on pyrolysis, contain amino acids and sugars (Zaika et al., 1968). However, there is no information available on quantitative changes occurring among these constituents under pyrolysis conditions. This study reports the effects

of heating on the components of diffusates of beef extracts and model systems containing the constituents of the diffusate, and the involvement of amino acids and sugars in the development of a characteristic roast aroma.

MATERIALS & METHODS

Materials

The preparations used included beef diffusates prepared from water extracts of beef muscle as described previously (Wasserman et al., 1965), model systems of amino acids and sugars in approximately the same concentrations in which they occur in beef diffusate and a mixture of solutions of amino acids and sugars to give a simulated beef diffusate.

Methods

4-ml samples of the various solutions, in 20-ml beakers, were heated for 30, 45 and 60 min in an air oven at 125°C. The volume in the beakers was great enough to ensure that liquid was still present in the sample at 30 min. Data for material analyzed at this time interval represent the composition of the solutions as they exist in the boiling state. At some point between 30 and 45 min the water completely evaporated and the 45-min samples were dry. The solid matter in the beakers was various shades of brown, depending on the contents. Very little change occurred during the next 15 min. The 45- and 60-min samples were considered to be pyrolyzed. Beakers were removed at appropriate times, the contents transferred to volumetric flasks and diluted to 10 ml with deionized distilled water. Since samples taken to dryness contained brown pigments that interfered with the analyses, all samples were treated with 0.5 g of a mixture of celite:charcoal (2:1) to remove color. Different amounts of amino acids and sugars are removed by adsorption of this mixture; therefore, all calculations are based on the concentrations present in similarly treated unheated samples. Investigation also showed that the concentration of component adsorbed was not dependent on its concentration in the solution; thus, changes found in components as a result of heating were not a reflection of the clarification process. The adsorbent material was removed by centrifugation and filtration through a Millipore filter. The colorless, clarified solutions were stored at -18°C until analyzed.

Decolorized solutions were analyzed quantitatively for amino acids by the Piez-Morris 1-column system (1960) using a Phoenix Analyzer. Total amino N was determined by the ninhydrin method of Cocking et al. (1954). The specific glucose oxidase technique was used according to the instructions of the Worthington Biochemical Corporation for analysis of glucose, and fructose analysis was carried out by the procedure of Bacon et al. (1948). The ribose determination using orcinol according to the method of Uehara et al. (1965) is not specific and must be corrected for concentrations of glucose and fructose if they are present in the solution.

The aromas of the solutions were characterized by several members of the laboratory familiar with the aroma of pyrolyzed beef diffusate. Differences in aromas were so obvious formal panel evaluation was not carried out.

RESULTS

THE AROMA of beef diffusate after 30

Table 1—Effect of heating at 125°C on the concentrations of amino acids and sugars in a diffusate of a water extract of beef.

	Heating time—min			
	0	30	45	60
	(μM/ml)			
Cysteic Acid	0.023	0.036	0.034	0.034
Taurine	3.868	3.602	1.534	1.399
Urea	6.818	5.217	3.022	3.335
Serine	0.554	0.551	0.345	0.322
Glutamic Acid	0.464	0.472	0.212	0.176
Proline	0.209	0.213	0.168	0.199
Glycine	1.078	1.074	0.417	0.363
Alanine	3.993	3.806	2.703	2.571
Valine	0.392	0.386	0.271	0.243
Methionine	0.098	0.098	0.042	0.041
Iso-leucine	0.257	0.204	0.145	0.137
Leucine	0.378	0.365	0.228	0.207
Ornithine	0.213	0.166	—	—
NH ₃	3.318	3.717	1.542	1.088
Lysine	0.171	0.159	0.083	0.054
Anserine	0.972	0.783	0.823	0.651
Carnosine	4.958	4.614	3.117	2.823
Asparagin + threonine	0.979	0.743	0.231	0.220
Arginine	0.243	0.097	0.097	0.092
Amino N	6.098	5.145	2.630	2.416
Sugars				
Glucose	5.503	5.584	0.044	0.009
Fructose	3.136	2.764	0.032	0.023
Ribose	1.066	0.833	0.013	0.010

min of heating was typically brothy; the 45- and 60-min samples had a characteristic burnt-meat aroma—sharp and acid.

The effects of heat treatment on the amino acid and sugar contents of beef diffusate are shown in Table 1. In the boiling state (the first 30 min of heating) only minor changes were observed except for the 60% decrease in arginine and the 57% increase in cysteic acid, probably as a result of cysteine oxidation. Large changes occurred on pyrolysis, as shown by the 45-min sample. There was a loss of 40–60% of most of the amino acids with a 77% loss of the asparagin-threonine complex being the greatest decline. Total amino N showed an average loss of 15% on boiling and a 60% decline after pyrolysis.

Glucose, fructose and ribose are present in beef diffusates. The phosphorylated forms of these sugars have also been identified (Zaika et al., 1968) but are present in such low concentrations that it was decided to investigate the changes in the parent sugars only. After 30 min of boiling the greatest loss was in ribose; glucose was apparently unaffected. This agrees with previous reports that of these 3 sugars the most reactive in the browning reaction is ribose, and glucose the least (Pomeranz et al., 1962; Casey et al., 1965). On pyrolysis, all of the sugars disappeared very rapidly as shown by the

45-min samples. There was very little further change in either amino acids or sugars on heating the beef diffusate the extra 15 min from 45 to 60 min.

There is a tendency to consider only the possible interaction between amino acids and sugars as the source of the brown pigments and odorous compounds that form on pyrolysis. However, Merritt et al. (1967) have shown that pyrolysis of amino acids alone resulted in the formation of odorous components, and many investigators have reported on the complex nature of the compounds formed on heating sugars (Hodge, 1967). It is possible that these reactions may be occurring simultaneously with, but independently of, amino acid-sugar interaction in diffusates.

An amino acid mixture containing these compounds in approximately the same concentrations found in beef diffusate was heated as just described. The aroma at 30 min was reminiscent of potato, but after pyrolysis there was no odor and no brown pigment formation. Amino acid changes are shown in Table 2. In general, there are only a few changes in amino acid concentrations in the 30-min sample; cysteic acid, since no cysteine was added to the system, declined 30%, glutamic acid declined about 25% and the methionine concentration was halved. On pyrolysis (45- and 60-min samples) some

Table 2—Effect of heating at 125°C on the concentrations of amino acids in a model system.

	Heating time—min			
	0	30	45	60
	(μM/ml)			
Cysteic Acid	0.087	0.062	0.061	0.063
Taurine	3.380	3.380	2.798	2.778
Urea	5.180	4.893	3.578	3.210
Serine	0.380	0.390	0.353	0.325
Glutamic Acid	0.318	0.243	0.200	0.205
Proline	0.803	0.748	0.720	0.728
Glycine	1.960	1.985	1.790	1.830
Alanine	3.140	3.193	2.920	2.983
Valine	0.318	0.310	0.285	0.288
Methionine	0.045	0.023	0.024	0.018
Iso-leucine	0.055	0.053	0.048	0.050
Leucine	0.230	0.220	0.150	0.148
Ornithine	0.188	0.243	0.230	0.118
NH ₃	2.500	2.353	1.835	1.943
Lysine	0.195	0.260	0.183	0.125
Anserine	0.083	0.275	0.245	0.253
Amino N	5.183	4.002	3.963	3.963

further losses were noted, but many amino acids were present in 80–90% concentrations of their initial levels. There was, however, a definite decrease in amino acid concentration as a result of pyrolyzing these compounds alone.

Results of heating individual sugar solutions, in approximately the same concentration in which they appear in the diffusate, at 125°C for various periods of time are shown in Table 3. After 30 min of heating the greatest loss, 26%, was in ribose; there was essentially no effect on fructose and glucose. On pyrolysis, larger amounts of sugar disappear, with ribose disappearing in greater quantity than fructose and glucose being least affected. Even after 60 min of heating there was no browning in any of the sugars and the only aroma was a slight caramel odor in glucose solutions. The lack of caramelization in dried samples may be because the temperature was not great enough.

The 2 model systems, amino acids alone and sugars alone, were combined to give a solution approximating the composition of beef diffusate. After 30 min of heating there was no particular aroma, but the pyrolyzed material in the 45- and 60-min samples developed a rich, sweet, grainy aroma. It was not like that of beef diffusate. The changes occurring on heating this model solution are shown in Table 4. Major losses were observed for taurine, lysine and arginine during the boiling phase, but after pyrolysis drastic changes occurred in concentrations of most of the amino acids. Between 80 and 90% of the cysteic acid, taurine, urea, glutamic acid and glycine disappeared; 50–60% of the remaining amino acids

Table 3—Effect of heating at 125°C on the concentrations of sugars in a model system.

	Heating time—min			
	0	30	45	60
	(μM/ml)			
Glucose	3.482	3.328	2.981	2.793
Fructose	2.287	2.220	2.044	1.240
Ribose	0.821	0.652	0.429	0.344

Table 4—Effect of heating at 125°C on concentrations of amino acids and sugars in a model system simulating beef diffusate.

	Heating time—min			
	0	30	45	60
	(μM/ml)			
Cysteic Acid	0.104	0.083	0.033	0.021
Taurine	4.276	1.392	1.235	1.198
Urea	10.249	8.057	1.955	1.182
Serine	0.470	0.456	0.171	0.136
Glutamic Acid	0.310	0.286	0.096	0.041
Proline	0.943	0.909	0.551	0.325
Glycine	2.438	2.237	0.669	0.487
Alanine	3.661	3.674	1.654	1.442
Valine	0.362	0.351	0.429	0.269
Methionine	0.048	0.036	—	—
Iso-leucine	0.065	0.068	0.038	0.031
Leucine	0.261	0.255	0.075	0.086
Ornithine	0.211	0.259	0.099	0.107
NH ₃	2.638	2.019	0.546	0.570
Lysine	0.231	0.128	0.182	0.154
Anserine	0.432	0.312	0.176	0.159
Carnosine	4.411	4.402	1.919	1.949
Asparagin + threonine	0.749	0.760	0.262	0.214
Arginine	0.136	0.076	0.061	0.071
Amino N	7.90	6.550	2.250	1.777
Sugars				
Glucose	3.552	3.372	—	—
Fructose	1.846	1.735	0.069	0.069
Ribose	4.379	2.614	0.185	0.019

also disappeared after 60 min of heating. Lysine and valine, with only 35 and 25% loss, respectively, were present in greatest concentration. The pattern of the disappearance of the sugars and amino N was similar to that observed with the diffusate: Glucose and fructose were relatively unaffected by boiling, whereas approximately 40% of the ribose disappeared. All of the sugars disappeared on pyrolysis. The amino N level at 60 min of heating reflects the greater amino acid loss in the model system, with lower values than those present in beef diffusate after the same heating period.

Although none of the sugars remain in both beef diffusate and its model system following pyrolysis, the loss of amino N was less complete, with 40% left in the

diffusate and 22% in the model. It was of interest to determine the effect of increasing the sugar concentration on the aroma produced and on the equilibrium of the system. To simplify the procedure, glucose alone was added to beef diffusate and model solutions in concentrations of approximately 5, 10, 25, and 70 × the amount found in the diffusate. The data in Table 5 report the changes in glucose and amino N after heating at 125°C for 60 min. On the assumption that the breakdown of glucose alone occurred independently of the sugar-amino acid interaction, even in the presence of amino acids, the loss due to glucose (column 2) was subtracted from losses in the diffusate and model systems. The values thus obtained (columns 5 and 6) presumably

Table 5—Effect of glucose concentration on the disappearance of glucose and Amino N on pyrolysis.

(μM/ml)									
Glucose loss						Amino N loss ²		μM Glucose μM Amino N	
1	2	3	4	5 ³	6	7	8	5/7 ⁴	6/8
Conc ¹	Gluc. alone	Gluc. + AA	Beef diff.	3-2	4-2	Gluc. + AA	Beef diff.		
1	---	---	4.07	---	4.07	---	11.73	---	0.35
5 ×	0.80	17.67	24.83	16.80	24.00	13.87	15.67	1.2	1.5
10 ×	11.37	42.33	54.87	30.87	43.50	15.43	20.20	2.0	2.1
25 ×	10.49	85.77	105.00	75.33	94.57	16.17	24.47	4.6	4.0
70 ×	146.87	---	238.63	---	91.77	---	22.83	---	4.0

¹Concentration based on glucose content of beef diffusate: 4.07 = μM/ml.

²Beef diffusate contained 22.23 μM/ml Amino N and the model system contained 16.47 μM/ml Amino N. The Amino N was calculated as leucine by the ninhydrin method.

³Columns 5 and 6 were obtained by subtracting column 2 (glucose alone) from columns 3 and 4.

⁴These values were obtained by dividing column 5 by column 7 and column 6 by column 8.

represent glucose disappearance due to reaction with amino acids. With increasing concentration of glucose the amount of sugar disappearing increased, reaching a plateau at some concentration between 10 and 25 × the initial concentration. The loss of amino N also increased with additional glucose, reaching 100% loss at some level between 10 and 25 × the initial sugar concentration. The amount of glucose apparently reacting with a mole of amino N was calculated (columns 9 and 10, Table 5). In the basic beef diffusate 0.35 mole of glucose reacted with 1 mole of amino N. Increasing glucose concentration appeared to result in an increase in the ratio, leveling off at a final value of about 4 moles of sugar reacting with each mole of amino N.

DISCUSSION & CONCLUSIONS

BOILING beef diffusate for 30 min produces changes in components that yield a brothy aroma. However, the only significant modifications in the amino acid and sugar contents are losses in arginine and ribose. (Complete arginine loss was reported by Thaler et al. (1963) in roasting coffee.) It is possible that, if indeed amino acids and sugars are involved in aroma formation, the smaller losses in iso-leucine, ornithine, anserine and asparagin + threonine might be important, because at this time the composition of the aroma is unknown and trace quantities of a high flavor-impact component could be present.

Large changes occur on pyrolysis of beef diffusate with the production of a variety of aromas progressing through a characteristic roast beef aroma to an undesirable acid, burnt odor. All of the amino acids enter into the reactions, with varying degrees of losses, although the

over-all participation results in a 60% loss of amino N. From the data it is difficult to determine whether there is any pattern to the loss in amino acids. None of the amino acids appears to be specifically related to the aroma produced.

The loss of amino acids in beef diffusate reported by Macy et al. (1964b) compares favorably, percentage-wise, with the losses observed in these studies for the 10 amino acids directly comparable. However, it should be noted that while their preparation, when heated in a boiling-water bath, developed a brothy odor and browned extensively, it is not clear whether their solution had evaporated to dryness or still contained water after the 60-min heating period. On the basis of amino acid loss it appears from both this study and that of Macy et al. that the changes are related to a condition in which there is little or no water and brown pigments eventually form. The main difference between the data reported here and those of Macy et al. is in the sugars; all of the sugars in this study were completely utilized, whereas a substantial concentration of glucose, in particular, remained in their preparation.

Amino acids alone break down to some extent on pyrolysis. Merritt et al. (1967) have shown that some of the breakdown products were similar to those obtained from the Strecker degradation. More important, they demonstrated with dipeptides that the components produced were dependent on the order in which the amino acids were linked. Thus, the use of model systems of individual amino acids may not give the desired information with respect to heating meat or meat extracts. Although no particular aroma was noted in pyrolyzed preparations, the contribution of this reaction must be

kept in mind in evaluating the over-all browning reaction products.

The presence of carbohydrate accelerated the breakdown of amino acids in both the beef diffusate and the model system. It is of interest to note that the breakdown was more complete in the model system; the diffusate may contain other components that have a protective action toward amino acids, or the form in which amino acids exist in the diffusate may hinder the reaction.

Although sugars did not brown under the conditions of our experiment, even when heated in the dry state, losses occurred. The rate of loss indicates that not only under browning conditions, as reported by others, but in pure solutions as well, the rate of decomposition, in decreasing order, is ribose > fructose > glucose. The significance of this rate of reaction is not immediately apparent except possibly in establishing the time sequence of the development of aromas. The interaction between sugars and amino acids is very evident in the formation of brown pigments and the increased reduction of sugar concentration in the presence of amino acids.

Glucose caramelization occurs even in the presence of amino acids. This can be deduced from the sweet, caramel aromas developed in the solutions containing large excesses of sugar. If it is assumed that caramelization, or sugar-sugar, reaction occurs simultaneously with sugar-amino acid reaction, correction for the former gives the approximate amount of sugar destroyed by reaction with amino acids. In beef diffusate about 0.35 mole of sugar reacts with a mole of amino N. The data of Macy et al., recalculated, indicate the glucose/amino N ratio is approximately 0.15, but not all of their

glucose was utilized in the reaction. At the level where molar concentrations of amino acid and glucose were approximately equal, the ratio was a little greater than 1.0. In solutions containing an excess of glucose the ratio of glucose/amino N disappearance was greater than 1.0, finally leveling off at a ratio of 4 moles of glucose used per mole of amino N. Reynolds (1965) reviewed the presence of diketose amines and di-D-glucosylamine in heated mixtures of sugars and amines, where 2 sugar molecules effectively fill the available active sites on the amino N. The fact that 4 moles of glucose will ultimately react with a mole of amino N suggests that the sugar moiety of a possible diglucosylamine is broken down and replaced. Reynolds (1965) also reported that in model systems of glucose and glycine, where the initial molar ratio was 8:1, the ratio of glucose lost to free amino groups lost reached 6:1. However, it is of interest to note that Wood (1961) reported the loss of 6 moles of amino acid per mole of sugar lost in a model system.

There is no doubt that the interaction of amino acids and sugars, or other reactive components, produces browning in diffusates of meat extracts, but there is still no identification of the components of the meat aroma formed or the mechanism of the reactions leading to their formation.

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